REMARKS

Claims 1-23 were pending.
Claims 1-23 are rejected.
Claims 1, 4, 7 and 13 are amended.
Claims 24 and 25 are new.

35 USC 112, second paragraph
Claims 1-23 are rejected under 35 USC 112, second paragraph.

Claim 1 has been amended to eliminate the phrase " characterized by a treatment system".

Claim 4 was previously amended to remove "preferably" in the last Applicant response. Applicants have further amended claim 4 to delete the reference to "treatment system" as this has been deleted in claim 1.

Claim 23 was previously amended to eliminate " and amino acids such as" in the last reply by the applicants.

Claim 7 is amended to replace "treatment system" with "solid-liquid separation stage".
Claim 14 is amended as in claim 7.

Applicants believe the above amendments correct all the 112, second paragraph rejections.

No new matter has been added.

35 USC 103(a)
Claims 1-3, 11, 20, 22 and 23 are rejected under 35 USC 103(a) as being unpatentable over Brink, US 5,536,325 in view of Minowa publication "the Characteristics of Dewatering Ethanol Fermentaion Stillage".

Examiner believes Brink to disclose a process for separating suspended solids from a fermentation liquor wherein the liquor removed from the distillation stage would comprise lignin.

Brink does not mention the specific treatment used in the separation stage. Examiner believes Minowa to disclose that it is known in the art to add cationic and anionic polymeric coagulants to aid in dewatering.

Applicants have amended claim 1 to read:

1.(currently amended): A process of separating suspended solids from a fermentation liquor by subjecting the liquor to a solids-liquid separation stage,
wherein the fermentation liquor is produced in a fermentation process for the production of a fermentation product,
which fermentation liquor comprises lignin,
wherein the solids-liquid separation stage ~~is charaterized by a treatment system which~~ comprises treating the fermentation liquor with an anionic polymer,
with the proviso that ~~the treatment system~~ solid-liquid separation stage does not include a cationic polymer having an intrinsic viscosity (IV) of at least 4 dl/g and the suspended solids from the fermentation liquor contain mainly lignin, with the proviso that when the anionic polymer is a synthetic polymer, the synthetic polymer has an intrinsic viscosity of at least 4 dl/g (measured in 1 M NaCl at 25° C).

Thus the suspended solids in the fermentation liquor presently claimed contain mainly lignin or greater than 50% lignin. While Brink may contain small amounts of lignin, Brink does not suggest suspended solids in fermentation liquors which are mainly lignin.

Furthermore, Minowa does not make up for this deficiency in Brink.

Minowa discloses the use of anionic coagulants for dewatering of fermentation stillage for rice and buckwheat. Both rice and buckwheat are carbohydrates containing little if any lignin.

Applicants attach pages from Ullmann Encyclopedia and several pages from "Roempp", a standard chemical encyclopedia in German to support this assertion.

Page 1, second paragraph from "Roempp" lists the composition of rice:
100g of unpolished rice contains on average

13.1 g water,
7.4 g proteins,
2.4 g lipids,
75.4 carbohydrates,
0.67 crude fiber,
1.2 g mineral nutrients and B vitamins.

Thus, lignin is not a significant component of rice.

As evident from Ullmann's encyclopedia, starch materials such as grains (buckwheat would fall into the category) also appear to contain little if any lignin. See sections 5.4.2 and 5.4.3 of Ullmanns describing the differences between starch and lignocellulosic materials.

Further, as stated in the present disclosure on page 7, lines 23-27 a fermentation liquor which contains mainly lignin solid residues has significant advantages:

> In addition when the fermentation liquor contains lignin the solid residues (mainly lignin), resulting from the separation process have higher cake solids than those recovered from conventional separations. Such a solid product would take less time and energy to dry and thus can be for instance used more efficiently as a solid fuel.

And finally, neither Brink or Minowa suggest using anionic polymers of intrinsic viscosity of at least 4 dl/g . As admitted by the examiner, the present claims differ from Brink by reciting the use of a specific treatment system in the separation stage. Minowa uses anionic coagulants. Coagulants are known by the art skilled to represent low molecular weight polymers.

Thus applicants submit that the combination of Brink with Minowa does not arrive at the presently claimed invention. While Brink is concerned with a fermentation liquor, the solid-liquid separation of the suspended solids in Brinks fermentation liquor does not comprise mainly lignin. The stillage of Minowa contains little if any lignin. Additionally, the anionic coagulants disclosed in Minowa for treating the fermentation liquor suspended solids, cannot be considered an anionic synthetic polymer of intrinsic viscosity of at least 4 dl/g. As a result, the combination of Brink with Minowa does not arrive at the claimed invention.

Claims 4-10 and 12-19 are rejected under 35 USC 103(a) as being unpatentable over Brink in view of Minowa (as above) and further in view of Moffett US 6,132,625.

Moffett, US 6,132,625 relates to a process of separating biosolids from an aqueous stream resulting from animal or vegetable processing operations using as flocculants an anionic inorganic colloid and a cationic polymer having a molecular weight greater than 1,000,000.

Examiner believes it to be obvious to one skilled in the art to modify the references (Brink and Minowa above as above) by addition of the recited polymers and siliceous material in view of the teachings of Moffett, to aid in dewatering solids in the separation stage.

Firstly, applicant directs the examiner to Moffett's description of the aqueous streams in col. 3 first paragraph.

In the process of this invention, the aqueous stream to be treated can be from any processing plant that produces an aqueous stream comprising biosolids, such as food processing plants. For example, animal slaughterhouses and animal processing plants and other food processing plants may produce aqueous streams comprising protein, fats and oil. Animal slaughterhouses and processing plants include those for cattle, hogs, poultry and seafood. Other food processing plants include plants for vegetable, grain and dairy food processing, for example, plants for processing soybeans, rice, barley, cheese, and whey; plants for wet-milling of starches and grains; as well as breweries, distilleries and wineries. Biosolids present in aqueous streams from these processes may include sugars, starches and other carbohydrates in addition to protein, fats, and oils. For example in processing of soybeans, proteins are extracted into an aqueous stream from which they are subsequently recovered. The present invention is especially useful for treating streams from animal processing, and more particularly, from poultry processing.

The above streams do not encompass suspended solids comprising mainly lignin. These biosolid aqueous streams are concerned with sugars, starches and other carbohydrates in addition to protein, fats and oils.

As shown in the Ullmann's encyclopedia description of readily fermentable carbohydrates, the suggested sources are quite different than lignocellulosic materials. Thus Moffett's aqueous streams do not contain mainly lignin as required by the present claims. Furthermore, the present claim limitations include a proviso that the solid-liquid treatment system does not include a cationic polymer having an intrinsic viscosity (IV) of at least 4 dl/g.

Moffett requires a cationic polymer of at least an average molecular weight greater than 1,000,000.See claims 1 and 2 of Moffett. The above presently claimed proviso excludes such cationic polymers in the solid-liquid separation stage wherein the suspended solids are mainly lignin.

As Moffett does not suggest fermentation liquors comprising lignin, Moffett does not make up for the deficiencies of Brink and Minowa. As Moffett absolutely requires the presence of a cationic polymer of at least an average molecular weigh greater than 1,000,000, and the present claims excludes such cationic polymers, claims 4-10 and 12-19 are unobvious in light of Brink and Minowa in light of Moffett.

Claim 21 is rejected under 35 USC 103(a) as being unpatentable over Brink, Us 5,536,325 in view of the Minowa et al. publication "the characteristics of Dewatering Ethanol Fermentation Stillage" as above, and further in view of Chieffalo et al. US 5,975,439.

Chieffalo as argued previously, relate to an automated process for producing ethanol shredding the cellulosic component of municipal solid waste and mixing this with equal amounts of concentrated sulfuric acid to provide a hydrolyzed mixture. At this stage, the solid by-product containing lignin is separated by filtration and the hydrolysate is subjected to fermentation. See col. 10, lines 49-53; col. 6, lines 40-49.

Therefore, the fermentation liquor of Chieffalo does not contain mainly lignin.

Claim 21 depends from claim 1 and thus carries all its limitations.

The examiner has relied on Brink in view of Minawa. These two references have been argued above and do not arrive at the presently claimed process (neither Brink or Minowa teach fermentation liquor containly suspended solids of mainly lignin and Minowa teaches only the use of anionic coagulant). The addition of Chieffalo does not make up for the deficiencies of Brink and Minowa as Chieffalo does not teach fermentation liquor which contains mainly lignin and has no teachings directed to solid-liquid separations using anionic polymers.

Double Patenting

Claims 1-23 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-19 or copending 10.523,301 and claims 1-22 of copending 10/587,583.

This is a provisional rejection. The Applicants request a delay in submitting a terminal disclaimer until all the other rejections have been overcome. At that time Applicants will know the state of the present claims and can reasonably evaluate the double patenting rejection.

Reconsideration and withdrawal of the rejection of claims 1-23 is respectfully solicited in light of the remarks and amendments *supra*.

Since there are no other grounds of objection or rejection, passage of this application to issue with claims 1-23 is earnestly solicited.

Applicants submit that the present application is in condition for allowance. In the event that minor amendments will further prosecution, Applicants request that the examiner contact the undersigned representative.

Respectfully submitted,

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Enclosures: Readily Fermentable Carbohydrates from Ullmann's Encyclopedia and two pages from "Roempp", a standard chemical encyclopedia in German.

5.4. Raw Materials and Processes

Raw materials for the production of ethanol by fermentation can be classified as:

1. readily fermentable carbohydrates that can be used directly, and
2. starch and other organic materials that must be converted to a fermentable form prior to fermentation.

The raw materials come from three major sources:

1. agricultural crops,
2. forest products, and
3. industrial and agricultural byproducts and residues.

Depending on need, end use, and availability, the choice of raw material varies for different regions, countries, and industries.

5.4.1. *Readily Fermentable Carbohydrates*

Various sugar crops, such as sugarcane, sugar and fodder beet, fruit crops, and crops based on crassulacean acid metabolism (CAM), are in this category.

Sugarcane. Sucrose [57-50-1] (α-D-glucopyranosyl- β-D-fructofuranoside) is the sugar obtained from cane or beet (⇒ Sugar). Sugarcane is a tropical crop whose successful cultivation is limited to an area spanning 37 °N to 31 °S.

Although sugarcane is grown mainly for production of table sugar and molasses, it is also an excellent raw material for the production of ethanol. The fermentable carbohydrates from sugarcane can be used either as the cane juice directly or as blackstrap molasses (a sugar byproduct). A material balance shows that 160 kg of fermentable solids can be obtained from 1 t of cane [263].

The cane juice is prepared by crushing raw cane and extracting the sugar with water, followed by clarification using milk of lime and H_2SO_4 to precipitate the inorganic materials [264]. The resulting extract is a green, sticky fluid, slightly more viscous than water, with an average sucrose content of 12 – 13 % [265].

Blackstrap molasses is the residue remaining after sucrose has been crystallized from cane juice. Molasses is a heavy viscous material, which contains sucrose, fructose, and glucose (invert sugar) at a total concentration of ca. 50 – 60 % (wt/vol) [266]. In contrast to cane juice, molasses is stable on storage and is usually diluted to the desired concentration just prior to fermentation.

A typical process for the production of ethanol from sugarcane is depicted in Figure 20. Production data are listed in Table 16. Ethanol production reaches a maximum after 14 – 20 h and then decreases until ca. 95 % of the available sugar is consumed. The process is usually batchwise, but some semicontinuous [232] and continuous operations [242], [267-269] are also used.

Table 16. Yields in production of ethanol from sugarcane [267]

	Alcohol, indirectly from molasses	Alcohol, directly from sugarcane juice
Sugarcane yield in 1.5 – 2-year cycle (south-central region), t/ha	63	63
Average sucrose yield (13.2 wt %),	8.32	8.32

t/ha		
Crystal sugar production, t/ha	7.0	
Final molasses or cane juice production, t/ha	2.21	66.2
Fermentable sugar, molasses, or juice, t/ha	1.32	8.73
Alcohol yield at 100 % global efficiency, kg/ha	675	4460
Alcohol yield at reasonable 85 % global efficiency, L per ton of cane	11.5	75
or in L/ha	730	4800

* Hectare (ha) = 10^4 m^2.

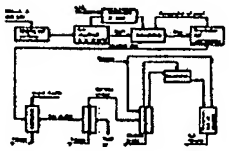


Figure 20. Typical process for the production of ethanol from sugarcane [267]

In the *batch process*, several fermentors are usually operating at staggered intervals to provide a continuous feed to the distillation columns. Overall productivity is ca. 18 – 25 kg of ethanol per cubic meter of fermentor volume per hour [225]. The "Melle Boinot" process is used in most Brazilian distilleries (see Section Batch Processes).

Ethanol has been produced in a *continuous process* (using continuously stirred tank reactors) from molasses by Danish Distilleries [268]. The process is shown in Figure 21, and performance data are given in Table 17.

Table 17. Performance data for the Danish Distilleries process * [268]

	Fermentor 1 (f_1) **	Fermentor 2 (f_2) **
Amount of yeast and dry matter per liter, g	10	10
pH	4.7	4.8
Alcohol, vol %	6.1	8.4
Residual sugar, %	1.0	0.1
Temperature, °C	35	35

* Residence time in each fermentor: 10.5 h; influx: 600 kg of molasses diluted in 22×10^3 L/h.
** See Figure 21.

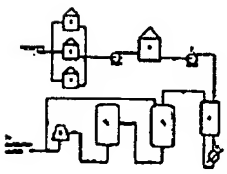


Figure 21. Continuous production of ethanol by Danish Distilleries [268]

a) Storage tank; b) Intermediate container; c) Metering pump; d) Regeneration section; e) Plate heat exchanger; f) Fermentor; g) Yeast separator

According to this process, the molasses is stored in two or three 1500-m^3 tanks from which it is pumped to intermediate containers. The material is adjusted for pH and nutrients (nitrogen and

phosphorus), sterilized at 100 °C by using plate heat exchangers, and then introduced to three fermentors with a total volume of 170 m^3. The fermented wort is centrifuged after fermentation, and the live yeast returned to the first fermentor. At the start, sufficient yeast propagation must be accomplished by aeration (0.02 – 0.03 L of air per liter of liquid per minute). The yield is ca. 28.29 L of alcohol per 100 kg of molasses, or a maximum of ca. 65 L of alcohol per 100 kg of fermentable sugar.

A continuous process for production of beer from sugar by use of *tower fermentors* is shown in Figure 22 [270], [271].

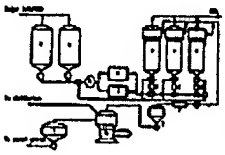


Figure 22. Commercial ethanol tower fermentation system (APV Company) [242]

a) Buffer storage tank; b) Divert line; c) Pasteurizer; d) Flow controller; e) Vertical cylindrical tower; f) Chiller; g) Yeast green beer buffer storage; h) Centrifuge; i) Separator

The key to the process is a vertical cylindrical tower fermentor with a conical bottom. A baffled yeast settling zone constitutes the upper part of the fermentor. The fermentor uses a flocculent yeast, which is pumped into the base of the tower. As the reaction proceeds, the beer rises, and the flocculating yeast settles and is retained in the reactor. High cell densities of 50 – 60 g/L are achieved without the use of mechanical cell concentration or separation devices. Short residence time (<4 h) with a sugar concentration of up to 12 % (wt/vol) sucrose, 90 % sugar utilization, and 90 % conversion to ethanol, produce up to 5 % ethanol in the final broth. The overall productivity of this system can be up to 80 times higher than that of the simple batch system.

Sugar Beet. Like sugarcane, sugar beet produces carbohydrates that consist primarily of sucrose (⇒ Sugar). Sugar beet is a more versatile crop than sugarcane. It can tolerate a wide range of soil and climatic conditions, and is grown throughout nearly half of the United States, Europe, Africa, Australia, and New Zealand.

In addition to sucrose, sugar beet contains sufficient nitrogen and other organic and microorganic nutrients [272] so that little, if any, fortification is required prior to fermentation. Another benefit is the high yield of coproducts such as beet tops and extracted pulp. The pulp has a high feed value, and the tops may be returned to the soil for erosion control and nutrient replacement. The yield of fodder beets is high (ca. 50 – 150 t/ha); their composition is described in [273].

A new fodder beet crop, produced in New Zealand through a genetic cross between sugar beets and marigolds, gives greater yields of fermentable carbohydrates per hectare than does sugar beet [274]. In addition, the sugar from fodder beet is reported to be more resistant to degradation over long storage.

Processes for the production of alcohol from sugar and fodder beets are basically the same as from sugarcane.

Fruit Crops. Many crops (grapes, plums, peaches, apricots, pineapples, etc.) contain variable proportions of sugars (sucrose plus fructose, usually 6 – 12 %). The fruit sugars can be readily fermented to alcohol, and this is done on a large scale for production of alcoholic beverages. The alcohol content of the product, which basically is the liquid after fermentation, separation of yeast, further treatment, and aging, depends on use and fermentation conditions. Table wines have <14 % alcohol, whereas wines with >14 % alcohol fall in the category of desert wines and aperitifs (⇒ Wine). Higher concentrations of alcohol are achieved by means of distillation to produce "strong" alcoholic beverages (e.g., brandy, whiskey, gin, vodka) (⇒ Spirits).

Alcohol for industrial or fuel use is seldom produced from fruit and vegetable crops. However, some fruit from tropical and semiarid climates, such as dates [275], mohwa flowers [276], and rain tree fruit [277], have been investigated for fuel alcohol production.

Crops Based on Crassulacean Acid Metabolism. Interest in using the agriculturally semi- or nonproductive regions of the world to grow alcohol-producing crops has increased [278]. These regions could be used to grow plants that utilize crassulacean acid metabolism (CAM) because their photosynthetic metabolism is extremely efficient with respect to irrigation requirements. These plants exhibit above-average productivity (expressed as a function of biomass production per unit of existing biomass) compared to other agricultural crops.

The CAM plants that are high in fermentable carbohydrates include various cacti (e.g., *Opuntia* sp.) and other plants such as *Euphorbia lathyrus* and *Agave* sp. Few data are available on potential ethanol production from these crops and its economic feasibility; however, an estimated 50 t/ha of these crops could be produced annually in subagricultural areas [279].

5.4.2. *Starch*

A variety of starch materials, such as grains, cassava, sweet potatoes, sweet sorghum, and Jerusalem artichoke, can be used for fermentation to ethanol (⇒ Starch). Selection depends on various factors, the major ones being climate and availability for large-scale production. Corn, wheat, potatoes, and Jerusalem artichokes are the most common raw materials in Europe and North America, whereas rice, cassava, sweet potato, and sweet sorghum are important in tropical countries.

Corn. Corn is the preferred raw material for conversion to alcohol in the United States and parts of Europe. It is available in large quantity, and its price (especially for low-grade or distressed corn) is thus acceptable for conversion to ethanol. Conversion to ethanol is efficient, and byproducts, such as corncobs, stalks, and leaves, are valuable as animal feed, energy source, or fertilizer. About 66 % of corn production is used for food and feed, and ca. 5 % is used to make alcohol (⇒ Cereals – Maize (Corn)).

A number of batch and continuous processes have been developed for production of ethanol from corn. A conventional fermentation plant producing 76×10^3 m^3 of anhydrous ethanol per year from 816.5×10^3 kg of corn per day is shown in Figure 23 .

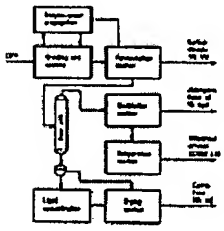


Figure 23. Flow diagram for a conventional fermentation plant producing anhydrous ethanol from corn [280]

In this process, corn is ground and cooked to dissolve and gelatinize the starch. The enzymes α-amylase and glucoamylase are then added to hydrolyze the starch to fermentable monosaccharides. After yeast fermentation for ca. 48 h at 32 °C, about 90 % of the starch is converted to ethanol. The fermentation broth is fed to the beer still where alcohol (ca. 50 vol %) is distilled. Subsequent distillation produces 95 % alcohol, which can be further concentrated by azeotropic distillation using benzene. After centrifugation, the stillage is concentrated to ca. 50 % solids in a multiple-effect evaporator, further concentrated in a fluidized-bed, transport-type dryer to ca. 10 % moisture, and then used as such for animal feed. This feed contains all the protein originally present in the grain, plus the additional protein from the yeast, resulting in a product containing 28 – 36 wt % protein.

In addition to alcohol and cattle feed, the original 816.5×10^3 kg of corn yields 175×10^3 kg of CO_2 and 95 kg of byproduct aldehydes, ketones, and fusel oils.

Alltech developed a method for an integrated grain-processing – fermentation route [281]. The grain pretreatment step prior to fermentation is shown in Figure 24.

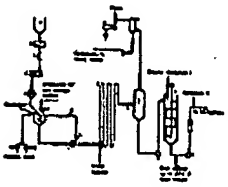


Figure 24. Alltech process for continuous whole mash cooking [281]

a) Grain hopper; b) Screen; c) Magnets; d) Continuous weigher; e) Hammer mill; f) Slurry vessel with agitator, temperature 50 – 70 °C; g) Rupture disk; h) Expansion vessel; i) Positive displacement pump; j) Continuous cooker tube, residence time 5 min, temperature variable up to 150 °C; k) Pressure valve; l) Flash vacuum cooler to 66 – 76 °C; m) Condenser; n) Open impeller pump; o) Converter, residence time 20 min, agitator 1 rpm; p) Wort cooler

Two enzymes, alcoholase I (from *Bacillus subtilis*) and alcoholase II (from *Aspergillus niger* and *Rhizopus niveus*) are used to hydrolyze the starch to fermentable sugars. Continuous whole mash cooking is applied. The ground starch is first mixed with water and alcoholase I at 60 °C, and then cooked at 93 – 165 °C in a batch or continuous cooker. The cooked mash is then cooled to ca. 66 – 76 °C, and a second portion of alcoholase I is added; 20 min is allowed for conversion. After this first hydrolysis step, the temperature is adjusted to 32 °C and the mash, supplemented with alcoholase II, is fermented with yeast.

Cassava. Cassava, also known as manioc, mandioc, aipum, yuca, cassada, and tapioca, is second in importance only to the sweet potato as a root crop throughout the tropics and in parts of South America where the plant originated. It was taken to West Africa by the Portuguese around 1914, where it now seems to have replaced yams and cocoyams because it adapts easily and requires less labor than other crops. Cassava is one of the highest yielding plants of the vegetable kingdom (10 – 30 t/ha); it requires little cultivation and the tubers can be left in the ground until required without serious deterioration.

Cassava (genus *Manihot*) is in the family Euphorbiaceae, which belongs to the subdivision Angiospermae, class Dicotyledoneae, order Geraniales. This large, widely spread family comprises 283 genera including 7300 species, with an almost worldwide distribution [282].

Manihot esculenta, *M. utilissima,* and *M. dulcis* are some economically important members of a genus which includes over 150 species that are distributed throughout tropical countries. The species include herbs, shrubs, and trees, many of them producing latex and some yielding rubber. Brazil, Indonesia, and Zaire are the largest producers of cassava.

Roots are generally of interest for alcohol production. They contain 20 – 35 wt % starch and 1 – 2 wt % protein, although strains with up to 38 % starch have been developed [283]. The advantages of cassava for fuel ethanol production (which can amount to 7600 L/ha) have been assessed [267], [284], [285]. The process used to obtain ethanol from cassava is shown in Figure 25.

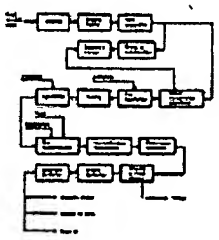


Figure 25. Production of ethanol from cassava root [267]

Fresh roots are washed, peeled, and ground into a mash. Part of this mash is dried; it can be stored in this form up to a year and is used for animal feed. For fermentation to ethanol, the starch is hydrolyzed with α-amylase, which is added in two steps. The first addition decreases the vicosity of the mash and facilitates cooking. In the second addition, the enzyme completes liquefaction of the starch. After that glucoamylase is added, which converts the liquefied starch to glucose and prepares the mash for fermentation. The fermentation process is the same as the one used for production of alcohol from sugarcane.

Alcohol yield from cassava is 165 – 180 L/t, which, on a mass basis, is higher than that obtained from sugarcane [285]. However, because sugarcane production can be as high as 90 t/ha, the alcohol yield per unit area is greater from cane under present cultivation conditions. Another advantage of cane is its dry fiber content, which equals the amount of total sugar present. This amount of fiber (bagasse) is sufficient to maintain the energy requirements of the plant; this is not the case with cassava, which only contains ca. 3.5 % fiber. Another disadvantage of cassava is that it does not contain readily fermentable sugars and, therefore, requires considerable processing of the roots prior to fermentation.

Sweet Sorghum. Sweet sorghum (*Sorghum sacchartum*) contains both starch and sugar. Its yield of ethanol from fermentable sugars is ca. 3500 – 4000 L/ha; an additional 1600 – 1900 L/ha can be produced from stalk fibers. There are more than 17 000 varieties of sorghum, and the yield is anticipated to increase by 30 % with some new hybrids [286]. The plant is adaptable to most of the world's agricultural regions; it is resistant to drought, and its nutrients are efficiently utilized by animals.

The fermentable sugars and starches are treated conventionally for ethanol production. The free sugars are fermented directly, whereas the starches are hydrolyzed by use of amylases, as is the case with cassava.

Potato. The potato is a common starch crop worldwide (⇒ Starch). The potato originated in South America (Chile and Peru) and came to Europe through Spain at the end of the 16th century. It is now grown in almost all climates and almost all types of soil, including dry and sandy soil [287].

Starch is the main carbohydrate component of potato (ca. 68 – 80 %). Depending on cultivation and variety of potato, starch content can vary between 12 and 21 % in raw potatoes. Only small quantities of soluble sugars are present (0.07 – 1.5 % sucrose, glucose, and fructose), as well as some rubber and dextrins (0.2 – 1.6 %) and pentosans (0.75 – 1.00 %).

The production of ethanol is based on fermentation of the available starch. A process developed by Danish Distilleries is shown in Figure 26 [268]. The process is semicontinuous and is applicable to both potatoes and grain.

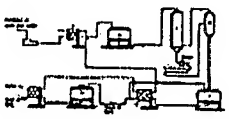


Figure 26. Danish Distilleries semicontinuous production of alcohol from potatoes or grain [268]

a) Preheater; b) Pulper; c) Enzyme treatment vessel; d) Flash cooler; e) Boiler tube; f) Holding tank; g) Condenser; h) Liquefaction vessel

Potatoes are mashed and then treated with amylases to hydrolyze the starch. The treatment section involves rapid steam treatment at 150 °C for ca. 3 min. The mash is cooled to 70 °C for liquefaction with commercial amylase preparations of bacterial origin; it is then cooled further to 30 °C and used for alcohol fermentation in the customary manner.

Jerusalem Artichoke. The Jerusalem artichoke (*Helianthus tuberosus*) is a member of the Compositae family and is closely related to the sunflower (*Helianthus annus*), earning it the nickname "wild sunflower". About 102 different names are synonymous with the name *H. tuberosus*.

The plant is native in North America. It was originally grown by the Cree and Huron Indians who called it askipaw and skibwan, respectively. The plant was introduced to Europe at the beginning of the 16th century where it rapidly spread through the Mediterranean countries. The addition of "Jerusalem" to the name is most likely the result of an English version of *Girasole*, the Italian name for this plant [288].

The plant grows 1.5 – 2.5 m tall for wild strains and up to 3.7 m under cultivation. Top growth accounts for 40 – 56 % of the total plant biomass. The tubers are of greatest interest as a raw material for fermentation to ethanol.

The main soluble carbohydrate in the Jerusalem artichoke is inulin, which is composed of a homologous series of polyfructofuranose units. These units consist of linear chains of D-fructose molecules joined by β-2,1-linkages. The chains are terminated by a D-glucose molecule linked to fructose by an α-1,2-bond as in sucrose [289].

A process to produce 360×10^3 kg/a of ethanol from Jerusalem artichoke tubers is presented in Figure 27 [290].

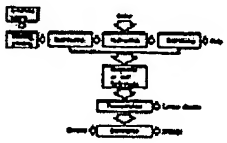


Figure 27. Production of ethanol from Jerusalem artichoke tubers [290]

In this process, the juice is expressed from the tubers and extracted with water to obtain a carbohydrate concentration of about 20 %. The carbohydrates (predominantly inulin) are then hydrolyzed enzymatically by activating the endogenous inulinases at ca. 50 – 60 °C; acid hydrolysis (pH ca. 1) of inulin is also effective. The resulting fermentable sugars are then converted to ethanol by a conventional route.

Novel routes for conversion of the juice to ethanol have also been explored; the flocculating yeast *Saccharomyces diastaticus* has been used in semicontinous and continuous modes [234].

5.4.3. *Lignocellulosic Materials*

Lignocellulose is the largest terrestrial source of biomass that is renewably produced through photosynthesis (⇒ Biomass Chemicals; ⇒ Cellulose – Cellulose; ⇒ Lignin). The solar energy reaching the earth surface is 3.67×10^{21} kJ/a [291]. Gobal photosynthesis (with an efficiency of 0.07 %) could convert 2.57×10^{18} kJ of that energy to cellulose-containing biomass. This would result in a net production of 1.8×10^{11} t/a of biodegradable material, 40 % of which is cellulose [292]. Estimates are that $1 - 1.25\times10^{11}$ t/a of terrestrial dry mass is produced together with $0.44 - 0.55\times10^{11}$ t/a in the oceans [293]. Present removal of this potential energy source is ca. 0.5 % of the total growing stock on a global basis [294].

The fermentation potential of lignocellulose is based mainly on the cellulose content of the biomass. Chemically, cellulose is similar to starch. It is a polymer of glucose in which the glucose units are linked by β-1,4-glucosidic bonds, whereas the bonds in starch are predominantly α-1,4-linkages. The degree of polymerization (DP) varies for different sources of cellulose; for example, newsprint cellulose has a DP of 1000, whereas cotton has a DP of ca. 10 000 [295].

The cellulose molecule is more resistant to hydrolysis compared to starch. This resistance is due not only to the primary structure based on glucosidic bonds, but also, to a great extent, to the secondary and tertiary configuration of the cellulose chain, as well as its close association with other protective polymeric structures such as lignin, starch, pectin, hemicellulose, proteins, and mineral elements.

The lignin molecule seems to be primarily responsible for difficulties in hydrolyzing the lignocellulosic material, because it forms a protective sheath around the cellulose microfibrils. Lignin is a macromolecule of phenolic character and can be viewed as a dehydration product of three monomeric alcohols: *trans*-4-coumaryl alcohol, *trans*-coniferyl alcohol, and *trans*-sinapyl alcohol. The relative amount of each varies with the source [296].

When cotton cellulose is treated with dilute acid, partial hydrolysis occurs rapidly, and ca. 15 % of the cellulose chain is degraded to glucose. The remaining 85 % is more resistant to hydrolysis, possibly because this portion of the cellulose exists in a highly crystalline order [295].

In order to use lignocellulosic materials for fermentation to ethanol, they must be pretreated and then hydrolyzed to fermentable sugars. Pretreatment may be physical or chemical, e.g., milling, steam explosion, or use of solvents and various swelling agents.

In *steam explosion* green wood chips are heated to ca. 180 – 200 °C for 5 – 30 min in a continuous operation (Stake process), or to a temperature of 245 °C for 0.5 – 2 min (Iotech process) [297]. The acids formed from hemicellulose under these high-temperature and high-pressure conditions start to "autohydrolyze" the cellulose and intact lignin. Lignin is sufficiently softened at the end of the steaming period, so that when the vessel is suddenly depressurized to atmospheric pressure, an explosion occurs within the woody cells. This partially disrupts the close association of cellulose with lignin and consequently increases the surface area available for further hydrolysis. The effect of steam pretreatment on the enzymatic hydrolysis of various cellulose-containing materials is shown in Table 18.

The pretreated lignocellulosic material is then subjected to further hydrolysis, which can be acidic or enzymatic (⇒ Cellulose – Cellulose; ⇒ Enzymes – Cellulases). A comparison of enzymatic and acid hydrolysis for cellulose degradation is given in Table 19. Cellulose and its degradation products are the only materials considered for fermentative purposes.

Table 18. Effect of steam pretreatment on the enzymatic hydrolysis * of cellulosic substrates [298]

Substrate	Pretreatment	Total reducing sugars, mg/mL	
		4 h	24 h
Hardwoods			
Poplar	none	1.4	2.4
	steam	15.3	25.8
Aspen	none	1.8	3.0
	steam	12.8	24.8
Agriculture residues			
Corn stover	none	4.9	7.8
	steam	15.7	22.5
Sugarcane bagasse	none	1.7	2.5
	steam	9.5	16.1
Urban waste	none	10.5	18.0
	steam	6.2	10.8
Softwoods			
Eastern spruce	none	2.0	3.8
	steam	3.5	6.4
Douglas fir	none	1.6	3.2
	steam	2.8	4.3

* *Trichoderma reesei* cellulase (QM9414), 19 IU (International Units) per gram of substrate; 5 % substrate slurries, pH 4.8, 50 °C; steamed substrates washed prior to enzymatic hydrolysis.

Table 19. Comparison between enzymatic and acid hydrolysis of cellulosic materials [227]

Acid	Enzyme

Nonspecific catalyst; therefore, will delignify material as well as hydrolyze cellulose	Specific macromolecular catalyst; therefore, extensive physical and chemical pretreatment of material necessary to make cellulose available for degradation
Decomposes hemicellulose to inhibitory compounds (i.e., furfural)	Produces clear sugar syrup ready for subsequent anaerobic fermentation
Harsh reaction conditions necessary; therefore, increased cost of heat- and corrosion-resistant equipment	Run under mild conditions (50 °C, atmospheric pressure, pH 4.8)
High chemical cost requires catalyst recovery and reuse	Cost of producing cellulases is the most expensive process step; therefore, recycle is necessary
High rate of hydrolysis	Lower rate of hydrolysis
Low overall yield of glucose because of degradation	High glucose yield depending on system and pretreatment

An example of a semicontinuous process for ethanol production from wood is shown schematically in Figure 28; this process uses dilute sulfuric acid for cellulose prehydrolysis.

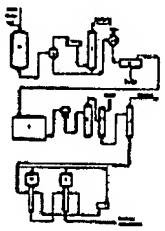


Figure 28. Ethanol production from wood [299]

Optimum conditions for this process are: acid concentration in total water, 0.53 %; maximum temperature of percolation, 196 °C; rate of temperature rise, 4 °C/min; percolation time, 145 –190 min; ratio of total water to oven-dried wood, 10; percolation rate, 8.69 – 14.44 L min^{-1} m^{-3}

a) Digester; b) Flash evaporator; c) Furfural tower; d) Neutralization vessel; e) Clarifier; f) Fermentor; g) Yeast separator; h) Alcohol stripper; i) Extraction tower; j) Rectifying tower; k) Evaporator; l) Vapor compressor

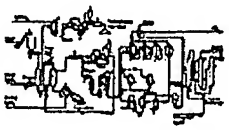
The hydrolysate percolates through a bed of wood chips. Optimum conditions for the process are described in [299]. After acid digestion (a), the effluent passes through a flash evaporator (b), which separates the vapors containing furfural and methanol from the underflow condensate containing the sugar solution. The acid hydrolysate solution is further neutralized with a lime slurry, and the precipitated calcium sulfate is separated in a clarifier (d) as a 50 % solids sludge. The neutralized liquor is blended with recovered yeast (*Saccharomyces cerevisiae*) from previous fermention and is fermented to ethanol (e), which is further concentrated to 95 % by distillation (i).

The bottom material from the alcohol stripping column (g), which contains pentose sugars, is further concentrated in multiple-effect evaporators (j) to a 65 % solution, that can be used as animal feed or for chemical conversion to furfural.

Figure 29 shows a strong acid hydrolysis process.

Figure 29. Ethanol production from wood by use of strong acid hydrolysis [300]

a) Feed hopper; b) Feeder; c) Digester; d) Neutralization vessel; e) Multiple-



effect evaporators; f) Dryer; g) Electrodialysis membrane; h) Filter; i) Fermentor; j) Carbon dioxide scrubber; k) Seed fermentor; l) Centrifuge; m) Yeast wash vessel; n) Surge vessel; o) Beer still; p) Alcohol column

The air-dry wood is first pretreated with dilute sulfuric acid (c). Complete hydrolysis is accomplished in a subsequent strong acid cycle in which cellulose is hydrolyzed at room temperature with 70 – 80 % H_2SO_4. The glucose, retained by the dialysis membrane (g), is neutralized, deionized, and then sent to fermentation (i). The sulfuric acid permeate from the dialysis unit is evaporated and reconcentrated for recycle. Lignin is separated from the concentrated acid by filtration (h) and washing.

To illustrate enzymatic hydrolysis of cellulose for alcohol production, a process used by the Natick Development Center is shown in Figure 30. A large part of this process involves the preparation of the cellulase enzyme. Newspaper is the cellulose-containing substrate.

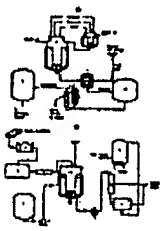


Figure 30. Enzymatic hydrolysis of newsprint by Natick Development Center (NDC) [298]

A) Pilot plant process for cellulase production:

a) Production vessel (vertical filters); b) Inoculum vessel; c) Filter; d) Harvest storage; e) Ultrafilter; f) Concentrate storageB) Pilot-plant process for newspaper hydrolysis: a) Ball mill; b) Solids metering; c) Solids transfer; d) Bioreactor; e) Enzyme storage; f) Metering pump; g) Harvest pump; h) Crude filter; i) Polish filter; j) Evaporator

5.4.4. *Waste Materials and Residues*

Various types of agricultural, industrial, or municipal refuse and waste can be used as substrates for ethanol fermentation. The fermentation is based on available sugar, starch, or cellulose in the waste material. The major advantage of this route lies in coupling waste treatment with the production of a higher value product. Both environmental pollution abatement and process economics are thus improved.

Cornstalks. Cornstalks are available in large quantities as a byproduct of corn agriculture. This material is predominantly composed of lignocellulose. A two-stage process using dilute acid treatment followed by concentrated acid impregnation of the lignocellulosic material is shown in Figure 31.

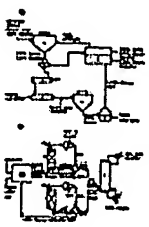


Figure 31. Production of ethanol from cornstalks [301]

A) Acid hydrolysis: a) Prehydrolysis tank, 4.4 % H_2SO_4; b) Filter; c) Rotary dryer; d) Impregnator; e) Hydrolysis tank, 8.0 % H_2SO_4; f) Filter; g) Electrodialysis unit

B) Fermentation: A) Acid hydrolysis process; h) Fixed film of *Fusarium oxysporum*; i) Centrifuge; j) Fixed film of *Saccharomyces cerevisiae*; k) Distillation column

In this process, ground corn stover (841 nm, 20 mesh) is treated with 4.4 % H_2SO_4 at 100 °C for 50 min (a). The mixture is then filtered (b) and the xylose-rich liquid is processed by electrodialysis (g) for acid recovery. The solids are dried further (c) and impregnated with 85 % H_2SO_4 (d), followed by dilution with water to give a H_2SO_4 concentration of 8 % (e).

Subsequent hydrolysis is carried out at 110 °C for ca. 10 min, and acid is again recovered by electrodialysis. The combined yield of xylose is 94 %, and the yield of glucose is 89 %. Glucose is converted to ethanol by *Saccharomyces cerevisiae* (j) and xylose by *Fusarium oxysporum* (h), both in immobilized cell reactors. The overall annual capacity of the plant is 17×10^3 m^3.

Domestic Refuse. Domestic refuse contains a complex variety of materials that come mainly from cellulosic-type residues. A large quantity of this material, ca. 1.3 – 2.2 kg per person, is generated daily. A process for the production of 36.5 t/d of ethanol from domestic refuse is illustrated in Figure 32 [302].

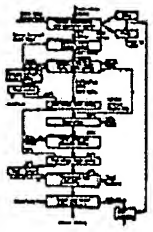


Figure 32. Flow diagram for continuous production of ethanol from refuse with 60 % content of cellulose [302]

* Biochemical oxygen demand.

The refuse is separated into dense and light fractions by the use of a flotation separator or a special pulper. The pulped fraction, which contains cellulose, is first subjected to removal of fines and plastics, and then introduced into a reactor where it is hydrolyzed with 0.4 % H_2SO_4 for ca. 1.2 min at 230 °C. This process is followed by flash cooling, neutralization with $CaCO_3$, and filtration. Fermentation of the sugar solution takes ca. 20 h at 40 °C and yields ca. 1.7 % aqueous ethanol solution, which is further concentrated by distillation to ca. 95 % ethanol.

Waste Liquor from the Pulp and Paper Industry. Two chemical pulping methods are predominant in the pulp and paper industry: the sulfate (Kraft) and the sulfite processes (⇒ Paper and Pulp). The basis of the these operations is treatment of the lignocellulosic material (wood, straw, etc.) with highly concentrated acid or base, which should dissolve the lignin portion of the wood and leave cellulose fibers that are processed into the final paper product. Depending on conditions (temperature, pressure, concentration of chemicals, chemical to wood ratio, and time of digestion), more or less delignification and breakdown of the original cellulose occur. As a result, a product pulp is produced as well as a waste chemical liquor, which basically consists of spent cooking chemicals. The more drastic the delignification conditions (low yield process), the better is the quality of the paper obtained. The high-yield process refers to milder delignification and a pulp that still contains a considerable amount of lignin. Low-yield processes are characterized by waste liquors with a high concentration of chemicals and a higher organic content.

The organic content of these liquors is primarily sulfonated lignin (e.g., 43 % of organic dry substance in a spent spruce sulfite liquor). However, cellulose and hemicellulose are also partially hydrolyzed during digestion so that waste liquors contain a certain proportion of free sugars (hexoses and pentoses in ca. 2 – 4 % concentration and ca. 14 % total solids) [303].

Tremendous quantities of waste liquor are generated in a pulp and paper mill; they amount to ca. 9180 L per ton of pulp produced [304]. Consequently, a chemical pulping process with a medium capacity of 500 t/d of pulp produces 4.59×10^6 L/d of waste liquors. Release of this liquor into natural waters is prohibited because both organic and toxic pollution result.

The sulfate (Kraft) process is designed so that the majority of the waste liquor can be recycled and its organic value converted to energy by combustion in a specially designed steam boiler (recovery furnace).

Previously, the majority of pulping mills worldwide were sulfite mills. Because of economic and environmental problems, sulfite pulping is gradually being phased out and the process converted to sulfate pulping or modified in other ways. Recovery of chemicals in the sulfite process is not as feasible as in the Kraft process, so large quantities of waste sulfite liquors

(WSL) are discharged to the environment.

Because WSL contain fermentable sugars, these liquors have been used efficiently as fermentation substrates for alcohol production. The process is relatively old (1908 in Sweden) but is still in operation in some mills (e.g., the Ontario Paper Company, Canada). A typical process for fermentation of WSL is shown schematically in Figure 33.

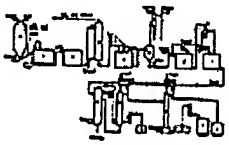


Figure 33. Production of ethanol from waste sulfite liquors (WSL) [305] *

a) Digester; b) Blowpits; c) Storage; d) Stripper; e) Screen; f) Storage; g) Flash cooler; h) Barometric condenser; i) Ejectors; j) Fermentor; k) Yeast separator; l) Storage; m) Preheaters; n) Beer still; o) Rectifying column; p) Oil washer; q) Fusel oil; r) Purifying column; s) Vaporizer; t) Condenser; u) Alcohol; v) Heads

* Reprinted with permission of American Institute of Chemical Engineers.

The waste liquor is first stripped of SO_2 with a conventional steam stripper. This is necessary because SO_2 would inhibit subsequent fermentation. The liquor is adjusted to give a ca. 10 – 12 % concentration of sugars, the pH is adjusted to 4.5, and nitrogen and phosphorus nutrient sources are added (e.g., urea and phosphate). The fermentation is conventionally carried out with yeast (*Saccharomyces cerevisiae*) at 30 °C for ca. 20 h. The yeast is usually concentrated and recycled, and the broth containing ethanol is sent to the distillation section.

Cheese Whey. Whey is a byproduct of cheese production (⇒ Cheese, Processed Cheese, and Whey – Introduction, Processed Cheese, and Whey). An estimated 74×10^6 t of whey is produced annually worldwide. This amount of whey contains ca. 0.7×10^6 t of milk protein and 3.2 t of lactose [306].

Whey with its protein, carbohydrate, and vitamin content is a valuable, nutritious material; its composition is described in [307]. Whey is used in various forms as a component of either animal or human food. However, the utilization and recycling of whey nutrients depend on many factors, one of which is the size of the cheese factory. The smaller the factory, the less recycling of whey is practiced.

The alcohol produced from whey is derived mainly from the fermentation of available lactose. However, only a few microorganisms can convert lactose to ethanol, and conventional yeast (*Saccharomyces cerevisiae*) is not among them. The most efficient lactose-utilizing organism is reported to be *S. fragilis* [308].

A process using *Kluyveromyces fragilis* yeast was developed in Denmark (Fig. 34) [309-311]. The whey is first concentrated by reverse osmosis and ultrafiltration, and then introduced into fermentation vessels. The yield based on lactose is about 80 % of theoretical. About 42 L of whey, containing 4.4 % lactose, is required to produce 1 L of absolute ethanol.

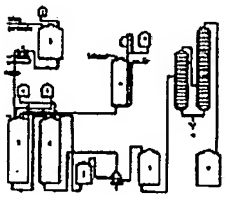


Figure 34. Continuous production of ethanol from whey [311]

a) Acid; b) Storage tank; c) Heat exchanger; d) Control; e) Antifoam; f) Chemicals; g) Fermentor; h) Substrate; i) Propagation plant; j) Storage; k) Separator; l) Buffer tank; m) Distillation; n) Alcohol storage

A better substrate for industrial fermentation of whey is enzymatically hydrolyzed lactose. β-Galactosidase-treated whey yields a mixture of monosaccharides, glucose, and galactose, which can be efficiently fermented by high-alcohol-producing yeasts [308], [312].

Reis

Stand: Mai 2005 > Bearbeitet von: Stefan Gräber
Fachgebiet: Biotechnologie und Gentechnik > Unterthema: Landwirtschaft (Pflanzen)
Fachgebiet: Lebensmittelchemie > Unterthema: Getreide und Getreideprodukte, Backwaren

(*Oryza sativa* L.). Zu den Gräsern (Poaceae) zählende, in tropischen und subtropischen Regionen vielfach kultivierte Getreideart. Das Hauptanbaugebiet für Reis (ca. 90% der Weltproduktion) ist Südostasien (China, Indien, Indonesien, Vietnam u.a.), aber auch die USA, Mexiko, Brasilien, Westafrika und Südeuropa sind Produktionsgebiete. Im Anbau unterscheidet man Sumpf- oder Wasserreis sowie Trocken- oder Bergreis. Die Weltjahresproduktion liegt bei 546 Mio. t und dient ca. 2,2 Mrd. Menschen als Grundnahrungsmittel.

Verarbeitung und Zusammensetzung:

Die nach dem Drusch vorliegenden bespelzten Körner (Roh- oder *Paddy-Reis*) werden zunächst entspelzt, wodurch man ernährungsphysiologisch wertvollen *Braunreis* erhält. Durch Schleifen und Polieren werden Frucht- und Samenschale (Silberhäutchen), der Keimling und die Aleuronschicht (vgl. Getreidekorn) entfernt. Diesen Reis bezeichnet man als *Weißreis*, wobei man je nach Korngröße und Form Rund-, Kurz-, Mittel- oder Langkornreis unterscheidet. 100 g unpolierter Reis enthalten durchschnittlich 13,1 g Wasser, 7,4 g Proteine, 2,4 g Lipide, 75,4 g Kohlenhydrate, 0,67 g Rohfaser, 1,2 g Mineralstoffe und B-Vitamine. Polierter Reis ist im Vergleich zu unpoliertem Reis sehr arm an Mineralstoffen und Vitaminen. Der ausschließliche Genuß von poliertem Reis führte bei Teilen der ostasiatischen Bevölkerung zur Beri-Beri-Krankheit (Thiamin-Avitaminose). Ein im Nährwert verbessertes Produkt wird durch den sogenannten Parboiling-Prozeß gewonnen.

Als wichtigste Aromakomponente von gekochtem Reis wurde 2-Acetyl-1-pyrrolin isoliert. Im Unterschied zu Europa und den USA sind in Asien Reissorten beliebt, die beim Kochen ein "popcornartiges" Aroma entwickeln. Es beruht auf der Bildung von 2-Acetyl-1-pyrrolin. Außer Vollreis oder Ganzreis (Ausbeute 45 –55%) fallen in der Reismüllerei Bruchreis bzw. Reismehl (20 –35%) und Spelzen bzw. Kleie (20 –24%) an.

Verwendung:

Langkornreis wird für Parboiled-Reis, Schnellkochreis sowie Dosen- und Suppenreis verwendet; Kurz- und Mittelkornreis werden dagegen für Trockengetreideprodukte, für Babynahrung und zur Bierherstellung verwendet. Rundkornreis ist als Milchreis im Handel. Vollreis wird zur Herstellung von Puffgetreide (Puffreis) und insbesondere in Ostasien zur Herstellung von Wein (Sake) und Schnaps (Arrak) benutzt. Da Reis keine Zöliakie-auslösenden Proteine enthält, kann er zur Zubereitung von Gluten-freier Diät verwendet werden. Bruchreis wird zu Grieß, Mehl, Stärke oder zu Reispuder (für Kosmetika) verarbeitet. Aus Reiskleie wird hochwertiges Kraftfutter oder Keimöl hergestellt. Die beim Schälen anfallenden Spelzen dienen als Verpackungs-, Heiz- und Isoliermaterial.

Gentechnik:

Reis besitzt ein kleines Genom (430 Mio. Basenpaare) und dient deshalb als Modellgenom für die Genomforschung an Getreide. Erfaßt werden möglichst viele Gene durch Ansequenzierung der entsprechenden cDNA; die Totalsequenzierung des Genoms wurde begonnen [1-3]. Reis ist relativ einfach transformierbar und Linien mit verschiedenen, gentechnologisch erzeugten Resistenzen (die jährlichen Verluste durch Insekten-, Virus- und Pilzbefall werden auf 45 – 75 Mio. t geschätzt) werden im Freiland getestet. Von besonderer Bedeutung sind Versuche, den Gehalt an Provitamin A im Endosperm des Reiskorns gentechnologisch zu erhöhen [4], um dadurch den in Südostasien noch weit verbreiteten Mangelkrankheiten vorzubeugen.

Übersetzungen:

E rice
F riz
I riso
S arroz

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